Genetic polymorphisms as determinants for disease-preventive effects of vitamin E

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Polymorphisms in genes involved in vitamin E uptake, distribution, metabolism, and molecular action may be important determinants for the protective effects of vitamin E supplementation. The haptoglobin 2-2 polymorphism is associated with increased production of oxygen free radicals and reduces levels of vitamin E and C; the consequent elevated risk for cardiovascular disease can be prevented by vitamin E supplementation.

INTRODUCTION

Atherosclerosis underlies important adverse vascular events, such as coronary artery disease, stroke, and peripheral artery disease, which are responsible for most of the cardiovascular morbidity and mortality. In the last two decades, numerous clinical studies have addressed the possible benefits of supplementation with vitamin E (α-tocopherol) and other micronutrients against atherosclerosis and other diseases such as cancer and neurodegeneration (reviewed previously[1–6]). Randomized clinical trials and epidemiologic studies with vitamin E supplementation intended to protect against cardiovascular disease (CVD) reported both positive and negative effects (reviewed previously[2]). Recent meta-analyses of the clinical studies even suggested there is increased all-cause mortality with high doses of vitamin E supplementation.[7,8] However, there is little evidence for adverse effects of vitamin E in adults when taken below the tolerable upper limit intake (1000 mg/day vitamin E according to the Food and Nutrition Board of the Institute of Medicine).[5] Vitamin E supplementation studies were generally aimed at reducing the amount of free radicals generated by inflammatory processes during disease development, and they were based on the findings that vitamin E levels in plasma and tissues can be increased by dietary supplementation where it chemically can act as an antioxidant.

Several factors have been proposed to explain the often null outcome of vitamin E supplementation in human studies, in particular, the relatively short duration of supplementation and the presence of high baseline levels of vitamin E in the normal diet sufficient to prevent disease symptoms.[9,10] Moreover, the dosage of vitamin E supplementation may have been too low, since a detectable reduction in the biomarkers of oxidative damage has only been achieved with much higher doses of vitamin E (>1600 IU) than those used in most primary and secondary prevention studies.[11,12] The potential health effects of vitamin E supplementation may become evident only under specific environmental and pathophysiological circumstances, such as local depletion of vitamin E by free radicals associated with inflammation, infection, smoking, or UV irradiation. However, patients with severe atherosclerosis display an imbalance of oxidant/antioxidant status in plasma that can be corrected by vitamin E supplementation, but with no effect on atherosclerotic plaques.[13] A recent study by Milman et al.[14] suggests that polymorphisms in specific genes, such as the haptoglobin gene, may increase the level of free radicals in diabetic patients and contribute to individual differences in response to vitamin E supplementation.

ROLE OF OXIDATIVE DAMAGE AND HAPTOGLOBIN GENOTYPE IN THE PATHOGENESIS OF ATHEROGENESIS

Oxidation of LDL (oxLDL) as a result of excess production of free radicals or their insufficient scavenging by antioxidant enzymes and redox-reactive micronutrients

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is recognized to play a central role in atherogenesis; however, a variety of other factors contribute to its development and progression (reviewed previously[15]). The oxidized lipids in oxLDL stimulate proliferation of vascular smooth muscle cells (VSMC) and accumulate in macrophages and VSMC, converting them to foam cells forming fatty streaks and atherosclerotic plaques. LDL oxidation is also catalyzed by hemoglobin through heme-initiated globin radical formation that can be prevented by irreversible binding of hemoglobin to haptoglobin, a plasma acute-phase α2-glycoprotein induced during inflammatory and neoplastic disease.[16–18] A higher plasma oxLDL/LDL ratio has been observed in individuals with the haptoglobin 2-2 polymorphism,[19] a genotype that occurs with a frequency of about 36% in Western societies.[20,21]

Hemoglobin (Hb), which is released by intravascular hemolysis of red blood cells, is a strong lipid oxidant through oxidation of its bound Fe^{2+} to Fe^{3+}.[18] Haptoglobin (Hp) is a plasma protein that binds with high affinity to hemoglobin forming a Hb-Hp complex, which prevents hemoglobin's oxidative damage (reviewed previously[22]). This complex is then recognized and degraded by the reticuloendothelial system via endocytosis through the hemoglobin scavenger receptor CD163, as well as by other receptors. In the context of cardiovascular disease, the CD163 receptor is expressed in monocytes and upregulated during their differentiation to macrophages; moreover, several cytokines such as interleukin (IL)-6 and IL-10 trigger induction of CD163 and of Hp (Figure 1).

In humans, the Hp gene exists mainly as two alleles Hp 1 and Hp 2, leading to haptoglobin 1-1, 1-2, and 2-2 genotypes. The magnitude of antioxidant activity of the Hp protein is measured to be in the order of Hp 1-1 > Hp 1-2 > Hp 2-2 > probucol > vitamin E.[25] The Hp-1-1 genotype is associated with resistance to the development of several diseases such as diabetic retinopathy, diabetic nephropathy, and CVD.[25,26] In addition to that, the expression level of the CD163 receptor on macrophages at sites of vascular injury[27] may play an important role in clearing the Hb released during plaque progression.

Since the Hb-Hp 1-1 complex is readily recognized by CD163 and more rapidly cleared, oxidative damage caused by hemoglobin in this complex may be low. In contrast, the Hp 2-2 protein binds with 10-fold higher affinity to hemoglobin, but this complex is less efficiently cleared, which may lead to oxidative damage to the vascular wall.[28] In this situation, supplementation with antioxidants such as vitamin E or C may show potent preventive effects. The formation of glycated hemoglobin and advanced glycation endproducts in diabetic patients may further accelerate the damage induced by non-degraded hemoglobin in the vascular wall.[21]

INFLUENCE OF GENETIC POLYMORPHISMS ON VITAMIN E-MEDIATED HEALTH BENEFITS

A recent, prospective, randomized, double-blind, placebo-controlled clinical trial by Milman et al.[14] found that middle-aged patients (1424 patients, ≥55 years) with type-2 diabetes and the haptoglobin 2-2 genotype show reduced cardiovascular events when supplemented with vitamin E (400 IU/d) for 18 months. These results confirm a previous association of the haptoglobin 2-2 phenotype and the preventive effects of vitamin E supplementation against myocardial infarction and cardiovascular death, which was obtained from reanalyzing data from the HOPE (Heart Outcomes Prevention Evaluation) study.[29] Interestingly, the recent findings of Milman et al.[14] are in contrast with the main conclusion of the original HOPE study, which failed to demonstrate the clinical benefit of vitamin E in patients with advanced CVD,[30] as well as with a follow-up study (HOPE-TOO) that reported no detectable preventive effect of vitamin E against cancer or major cardiovascular events in patients with vascular disease or diabetes mellitus.[31] Thus, as exemplified by the study of Milman et al.,[14] the preventive effects of vitamin E supplementation may depend on the presence of polymorphisms of specific genes involved in the production and scavenging of reactive oxygen and reactive nitrogen species and maintaining the balance within the antioxidant network.

In addition, polymorphisms in genes involved in the uptake, distribution, metabolism, and secretion of antioxidant micronutrients may play an important role in micronutrient bioavailability. For example, a number of genetic and epigenetic polymorphisms (that can occur in the homozygote or heterozygote state) may lower the bioavailability and cellular activity of vitamin E, which could be circumvented by vitamin E supplementation (Table 1). As reviewed by Rigotti,[72] several proteins participate in the uptake, distribution, and metabolism of vitamin E, and polymorphisms in these proteins and their cellular expression levels may explain individual differences in vitamin E uptake and response, thereby influencing a differential susceptibility to disorders such as atherosclerosis, certain cancers, and neurodegenerative diseases.[44] For reasons as yet unknown, patients respond differently to supplemented vitamin E, and they achieve different levels of plasma vitamin E after supplementation.[73] These differences could be the consequence of gene defects or polymorphisms resulting in changes in vitamin E transport efficiency, the rate of metabolism, the structure and plasma levels of lipoproteins, the status of other micronutrients involved in α-tocopherol protection, as well as some environmental factors.[73] Furthermore, it appears possible that polymorphisms in the postulated kinases, which are required for the synthesis of the more active

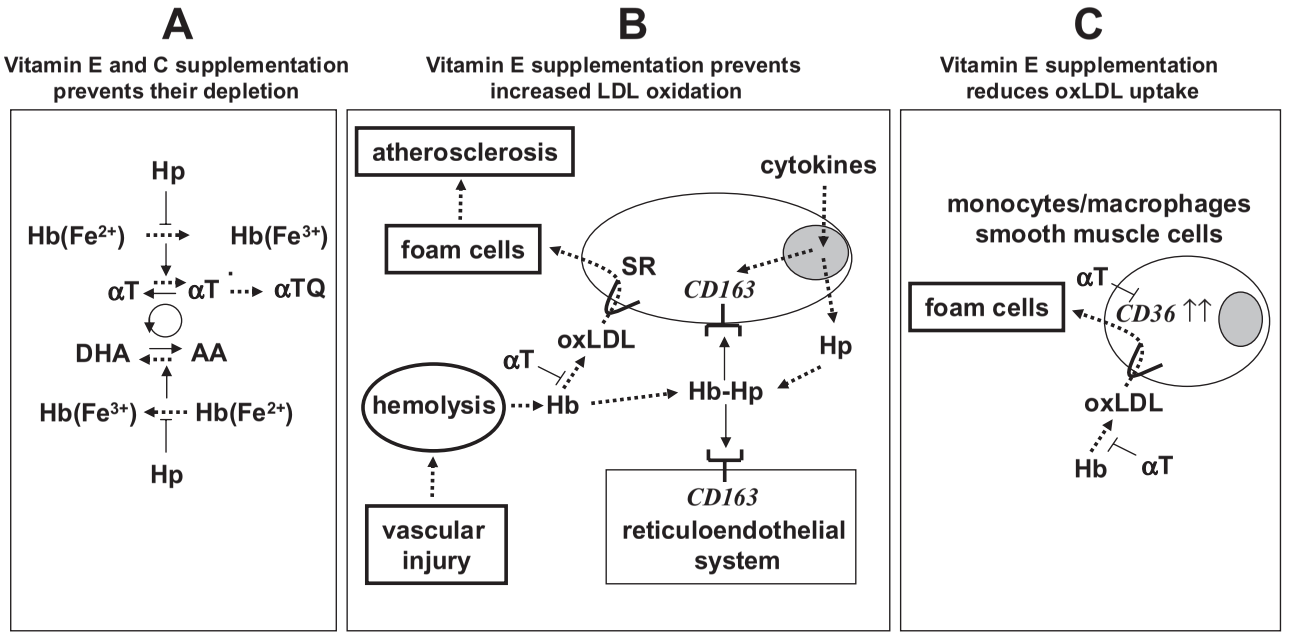

Figure 1 **Preventive action of vitamin E against cardiovascular disease in subjects with the haptoglobin 2-2 genotype.**

A. Depletion of vitamin E and C in subjects with the Hp 2-2 genotype. Hemoglobin (Hb) oxidation leads to depletion of vitamin E (αT) and C (AA) that can be prevented by haptoglobin (Hp). Hp 2-2 is less efficient than Hp 2-1 or 1-1 in preventing the oxidation of Hb; supplementation of subjects with the Hp 2-2 genotype prevents vitamin E and C depletion, restores the cellular functions of these vitamins, and reduces the formation of oxidized lipoproteins (oxLDL). Oxidation of vitamin E and C may be increased in subjects with diabetes or inflammation (stippled arrows). DHA, dehydroascorbic acid; αTQ, α-tocopheryl quinone

B. Increased oxidation of LDL in subjects with the Hp 2-2 genotype. Hemoglobin released during vascular injury and hemolysis increases the formation of oxLDL. Uptake of oxLDL by macrophages by the scavenger receptors (SR) can convert them to foam cells. Alternatively, released Hb is bound by Hp and the complex removed and inactivated via the CD163 receptor by macrophages or the reticuloendothelial system. Hp 2-2 is less efficient than Hp 2-1 or 1-1 in removing Hb; increased formation of oxLDL in subjects with the Hp 2-2 genotype can be prevented by supplementation with vitamin E and C. Oxidation of LDL may be increased in subjects with diabetes or inflammation (stippled arrows).

C. Increased uptake of oxLDL in subjects with the Hp 2-2 genotype. When the formation of oxLDL is a strong determinant for the development of atherosclerosis, such as in subjects with the Hp 2-2 genotype, overexpression of scavenger receptors on monocytes/macrophages and vascular smooth muscle cells (VSMC) may increase the formation of atherosclerotic foam cells. Vitamin E reduces the uptake of oxLDL by antagonizing the induction of the CD36 scavenger receptor by oxLDL on monocytes/macrophages and VSMC.[23,24] Oxidation of LDL and CD36 expression may be increased in subjects with diabetes or inflammation (stippled arrows).

Table 1 **Genes possibly affecting vitamin E bioactivity with respect to CVD.**

Candidate gene	Function in relation to vitamin E	Possible effects on vitamin E action when mutated or polymorph	Reference
Haptoglobin (Hp)	Increased levels of free radicals and depletion of vitamin E and C	Increased free-radical production with the haptoglobin-2-2 genotype	Langlois et al. (1997)[32] Brouwers et al. (2004)[19] Milman et al. (2008)[14]
Apolipoprotein E (ApoE)	Increased levels of free radicals and depletion of vitamin E and C, plasma lipoprotein turnover, vitamin levels in plasma, and tissue	Polymorphisms in ApoE, such as ApoE4, ApoE3, or ApoE2 are known to generate increased oxidative stress (apoE4 > apoE3 > apoE2). ApoE determines the uptake of HDL vitamin E into peripheral tissues via binding to SR-BI, ApoE4 genotype is associated with a lower tissue level, but an increased plasma level of vitamin E	Peroutka et al. (2000)[33] Gomez-Coronado et al. (2002)[34] Mardones et al. (2002)[35] Lodge et al. (2004)[36] Borel et al. (2007)[37] Jofre-Monseny et al. (2007)[38] Jofre-Monseny et al. (2008)[39]
Apolipoprotein A (ApoA)	Plasma lipoprotein turnover, vitamin E levels in plasma and tissues	Polymorphisms in ApoA, such as ApoA-IV may affect the level of γ-tocopherol in plasma and tissue	Borel et al. (2007)[37]
SR-BI scavenger receptor	Vitamin E uptake and transport to peripheral tissues	Polymorphisms in SR-BI may influence vitamin E levels in peripheral cells and tissues	Borel et al. (2007)[37]
CD36 scavenger receptor	Vitamin E downregulates the expression of CD36 with consequent reduced foam cell formation	Polymorphisms in the CD36 gene (promoter polymorphisms, alternative splicing) may influence responsiveness to vitamin E	Ma et al. (2004)[40] Zingg et al. (2002)[41] Andersen et al. (2006)[42] Cheung et al. (2007)[43]
LDL-receptor	Removal of LDL form plasma, vitamin E uptake	Polymorphisms may influence plasma lipid profile and possibly vitamin E levels in plasma and tissues	Doring et al. (2004)[44] Davis et al. (2005)[45]
Phospholipid transfer protein (PLTP)	Exchange of vitamin E between lipoproteins	Polymorphism in PLTP may influence the level of vitamin E in different lipoproteins (VLDL; LDL, HDL, chylomicrons)	Kostner et al. (1995)[46] Jiang et al. (2002)[47]
Microsomal triglyceride transfer protein (MTTP)	Incorporation of vitamin E into chylomicron	Polymorphisms in MTTP may influence the uptake efficiency of vitamin E	Anwar et al. (2007)[48]
Tocopherol-associated proteins (TAP1, TAP2, TAP3)	Vitamin E binding, uptake, and intracellular transport influence possibly lipid-dependent signal transduction and gene expression	Polymorphisms in TAP may influence intracellular and tissue levels of vitamin E as well as its cellular activity	Kempna et al. (2003)[49] Doring et al. (2004)[44] Ni et al. (2005)[50] Neuzil et al. (2006)[51]
α-Tocopherol transfer protein (α-TTP)	Vitamin E retention in plasma by liver α-tocopherol salvage pathway	Polymorphisms in α-TTP may influence plasma and tissue level of vitamin E	Gotoda et al. (1995)[52] Ouahchi et al. (1995)[53] Hentati et al. (1996)[54] Doring et al. (2004)[44]
Afamin	Vitamin E transport in cerebrospinal liquid and brain	Polymorphisms in afamin may influence levels of vitamin E in the nervous system	Voegele et al. (2002)[55]

Table 1 **Continued**

Candidate gene	Function in relation to vitamin E	Possible effects on vitamin E action when mutated or polymorph	Reference
Lipoprotein lipase (LPL)	Vitamin E transfer from lipoproteins into peripheral tissues	Polymorphisms of LPL may influence plasma and tissue levels of vitamin E	Traber et al. (1985)[56] Traber et al. (1992)[57] Doring et al. (2004)[44]
ATP binding cassette transporter A1 (ABCA1)	Vitamin E export from cells	Polymorphisms in ABCA1 may influence cellular and tissue levels of vitamin E	Oram et al. (2001)[58] Mustacich et al. (2006)[59] Frikke-Schmidt et al. (2008)[60]
Pregnane X receptor (PXR)	Vitamin E-mediated gene expression	Polymorphisms may influence vitamin E-dependent gene expression of PXR target genes	Landes et al. (2003)[61] Doring et al. (2004)[44]
Multidrug resistance protein (MDR2) P-glycoprotein	Involved in biliary secretion of vitamin E	Polymorphisms in MDR2 may influence levels of vitamin E and enterohepatic circulation	Mustacich et al. (1998)[62] Doring et al. (2004)[44]
P450-cytochromes (Cyp3A and Cyp4F2)	Vitamin E metabolism	Polymorphisms in metabolic genes may influence plasma and tissue levels of α-, β-, γ-, and δ-tocopherols, as well as the level of their metabolites	Birringer et al. (2001)[63] Sontag et al. (2002)[64] Doring et al. (2004)[44] Mustacich et al. (2006)[59] Abe et al. (2007)[65]
Dehydroascorbate reductase, e.g., omega class glutathione transferases (GSTO1-1 or GSTO2-2)	Regeneration of vitamin C and thus regeneration of vitamin E	Polymorphisms in GSTO1-1 or GSTO2-2 may influence plasma and tissue levels of vitamin C, and thus of vitamin E	Withbread et al. (2005)[66]
Sodium-coupled vitamin C transporters 1 and 2 (SVCT1/SLC23A1, SVCT2/SLC23A2) or dehydroascorbate transporters (GLUT1, GLUT3)	Regeneration of vitamin E by vitamin C	Polymorphisms in SVCT1 or SVCT2 may influence the plasma and tissue level of vitamin C, and thus that of vitamin E	Hediger (2002)[67] Na et al. (2006)[69] Seno et al. (2004)[70] Erichsen et al. (2006)[71]

α-tocopheryl phosphate or the phosphatases, which are required for hydrolyzing it, may play a role in determining the cellular activity of vitamin E.[74–77]

POTENTIAL BENEFITS OF COMBINED ANTIOXIDANT THERAPY

Vitamin E supplements may have to be coadministered with vitamin C (L-ascorbic acid) to effectively lower the amount of free radicals.[78–80] In fact, several prevention studies indicate that the combination of vitamins E and C is more potent in preventing atherosclerosis,[81–84] and it remains to be determined whether subjects with the Hp 2-2 genotype benefit even more when both vitamins are supplemented together. Vitamin C present in plasma and in the arterial wall efficiently prevents vitamin E oxidation and improves the myocardial and endothelial functions,[85,86] indicating that an intact cellular antioxidant network is important for maintaining cellular homeostasis.[87,88]

On the other hand, vitamin C may have a prooxidant activity by facilitating redox-cycling of free iron and increasing hemoglobin-iron-driven peroxidation. Thus, polymorphisms of L-ascorbic acid binding proteins involved in uptake, tissue distribution and metabolism, and their cellular expression levels could contribute to the individual vitamin E response (Table 1).[66–71] In line with this, serum vitamin C concentrations were lowest in serum from subjects with Hp 2-2, whereas other endogenous antioxidants (uric acid, bilirubin, albumin, ceruloplasmin, and total antioxidant status) were not different,

most likely resulting in a lower efficiency of regeneration of vitamin E by vitamin C in these subjects.[32,69]

At present, the effects of vitamin E supplementation in diabetic patients with the haptoglobin 2-2 genotype are mainly explained by its antioxidant action, but other effects of vitamin E supplementation cannot be excluded. Decreased levels of vitamins E and C as a result of oxidative utilization in subjects with the Hp 2-2 genotype most likely also affect alternative non-antioxidant mechanisms resulting from the modulation of cellular signaling and gene expression by interacting with and regulating specific enzymes and transcription factors or influencing cellular structures, such as membranes and lipid domains (reviewed in[73,89–91]). Hp 2-2 in the Hb-Hp-complex may induce a different cellular response when compared to Hp 1-2 or Hp 1-1, and it can be speculated that the higher preventive effects of vitamin E may also result from the modulation of Hb-Hp 2-2-induced and CD163-mediated signal transduction in antioxidant or non-antioxidant manners.

CONCLUSION

According to the study of Milman et al.,[14] clear preventive effects of vitamin E are only seen after selection of a patient group with the Hp 2-2 genotype and diabetes mellitus, which is characterized by increased production of oxygen free radicals. Compared to previous clinical trials with vitamin E supplementation, these findings suggest that confounding factors, such as polymorphisms in the Hp 2-2 genotype, are insufficient to increase the statistical power of studies to demonstrate a positive outcome effect of vitamin E supplementation, even though the prevalence of the Hp 2-2 genotype is 36% in the general population. Moreover, as shown with the HOPE-TOO study,[31] even when focusing on diabetic patients, the Hp 2-2 allele, which occurs together with diabetes in only 2–3% of the general population, did not shift the overall outcome of this large clinical trial.

In view of these findings, it is likely that additional mutations and polymorphisms in those genes known to modulate vitamin E absorption, distribution, transport, activity, metabolisms, and excretion, will be found that could shift the balance in the redox-active cellular network and generate low vitamin E bioavailability, resulting in similar deficiency syndromes with possible influences on atherosclerotic and associated cardiovascular events that can be prevented by additional vitamin E supplementation. Moreover, species-specific polymorphisms and gene variants may help explain why animal studies of vitamin E supplementation generally show more positive outcomes against atherosclerosis in many experimental settings, such as the clearer beneficial effects of vitamin E supplementation seen in vitamin E-deficient animals[92] or in animals having specific mutations, such as in knockout mice for the apolipoprotein E gene ($ApoE^{-/-}$), the low-density lipoprotein receptor ($LDL\text{-}R^{-/-}$) or the α-tocopherol transfer protein ($\alpha\text{-}TPP^{-/-}$).

Whereas the concept of individualized medicine with drugs designed specifically against disease-causing molecular targets is currently well accepted, the data from the haptoglobin polymorphism and vitamin E study by Milman et al.[14] provide a stronger scientific basis to help establish guidelines for recommending individualized supplementation with micronutrients based on polymorphisms in specific genes.

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